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NUTRITION REVIEWS

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NUTRITION REVIEWS

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HYPERTENSION AND NUTRITION

Hypertension is an important disorder closely connected with diet. Some clinical associations are the following.

Body weight is a recognized factor, to the extent that some of the milder elevations of pressure are reduced, even to normal levels, by simple reduction of obesity. Unselected hospital autopsies indicate that arteriosclerosis (with or without hypertension) practically parallels the body weight (S. L. Wilens, *Arch. Int. Med.* **79,** *129 (1947)*). On the other hand, weight reduction has negligible influence on really severe hypertension. Also etiologically, hypertension occurs in both thin and fat persons, and many extremely obese persons keep normal blood pressure indefinitely. The rational interpretation is that excess weight merely imposes an added circulatory burden in persons predisposed to hypertension.

Hypertension may occur in short and stout persons and in tall and thin persons. Also, its occurrence in both nervous and placid persons should not be confused by the fact that hypertension may cause nervousness, or an existing hypertension may be aggravated by nervous stress. These facts, together with the therapeutic palliation by rest, have given hypertension a prominent place in "psychosomatic" medicine. The true etiologic status of nervous or psychic excitement or depression may be judged by the multitude of nervous persons who are not hypertensive, and especially by the infrequency of hypertension among the insane.

The more direct relation between hypertension and diet involves a question of two factors, namely, protein and salt. In this brief review the liberty has been taken of dismissing the once generally accepted influence of protein. Kempner's recent revival of this anachronism cannot controvert the long series of researches proving conclusively that neither red meat nor any other form of protein has any influence, aside from its incidental salt content, upon normal or abnormal blood pressure.

Salt is the principal focus of present debate. This presentation will be limited to an outline of personal research and experience, with the principal supporting evidence.

In the first years of this century, the Widal-Ambard school (L. Ambard and Beaujard, *Arch. gén. de méd.* **81,** *520 (1904)*; Ambard, *Sem. méd.* **26,** *361 (1906)*) proposed salt restriction for hypertension, as an extension of their treatment of edema. This method failed of acceptance, partly for the practical reason that the exclusion of salt was not sufficiently thorough, but chiefly because their basic theory of chloride retention as the cause of hypertension was easily disproved. Therefore, by 1920, this treatment was not in use or even mentioned in textbooks, except to some extent in France.

Our own work was not prompted by the French suggestion but developed from observation of a case of congestive heart failure with severe edema, for which the rational treatment appeared to be rigid exclusion of salt. The improvement of the associated hypertension was so striking that the treatment was extended to other hypertensions without edema. Our preliminary report (F. M. Allen, *J. Am. Med. Assn.* **74,** *652 (1920)*) was based on 20 cases. The main publication (Allen and J. W. Sherrill, *J. Met. Res.* **2,** *429 (1922)*) comprised detailed studies of 180 cases during four years.

A practical manual (F. M. Allen, "Treatment of Kidney Diseases and High Blood Pressure," *Physiatric Institute, Morristown, N. J. (1925)*) contained detailed diet instructions which will be referred to later. Also several short papers were published (Allen, *Med. Clinics North America* **6,** *475 (1922)*;

J. Indiana State Med. Assn. **18,** *445 (1925); Illinois Med. J.* **49,** *401 (1926); J. Med. Soc. New Jersey* **23,** *543 (1926)*), one of them (Allen, *New York State J. Med.* **25,** *726 (1925)*) enlarging the series to 328 cases during six years, with the same durable benefits. As stated in a recent summary (Allen, Discussion of paper by D. Ayman, Section on Internal Medicine, *American Medical Association, June 9, 1949*), this work "included the first large statistics of dietotherapy in hypertension, the first adequately strict exclusion of chloride, bicarbonate and other sodium salts, consequently the first account of salt-deficiency symptoms and precautions against deaths such as recent authors report, the first classification of refractory cases and notice of ultimate benefit in many of them, the first recommendation for retinitis, eclampsia and congestive heart failure, and the first clear concept distinguishing symptom and specific disease controllable through a specific factor."

This work was not generally accepted by the medical profession, and denials appeared, based on brief observations of a few patients in the large clinics (F. M. Allen, *Boston Med. Surg. J.* **189,** *810 (1923); J. Med. Soc. New Jersey* **27,** *126 (1930); J. Am. Med. Assn.* **136,** *202 (1948)*). For many years lax "salt-free" diets containing 2 to 4 g. of sodium chloride continued to be used therapeutically and with generally disappointing results. More recently with adoption of strict low salt diets furnishing no more than 1 g. of salt per day, therapeutic benefits similar to those previously reported have been observed. Laboratory control is indispensable for accuracy. Actual sodium analyses are not feasible except in specially equipped institutions, but fortunately a simplified method permits adequate checks of the chloride excretion by any practitioner (J. M. Bryant, V. Iob, G. L. Phillips, and E. E. Blecha, *Ibid.* **140,** *670 (1949)*).

At present, opinions are divided on the therapeutic effectiveness of diets that are really low in salt, that is, furnish less than 1 g. of sodium salts daily. Superficial conclusions are drawn from brief observations of miscellaneous methods of artificially raising or lowering blood pressure. Some clinicians are finally grasping the concept of essential hypertension as a specific disease which happens to be named from its most prominent symptom. Of these, some uphold a fixed evolution of the disease regardless of diet, which they therefore ignore in treatment. Others admit a symptomatic benefit of salt restriction in some cases.

Evidently the majority of physicians now limit salt more or less strictly in hypertension. The popularization of the idea and the emphasis on precise exclusion of salt must be credited largely to Kempner's dramatic reports of benefits. His fixed ritual has been helpful for guiding many physicians, but the predilection for rice and the ban on protein and condiments are scientifically unfounded, and in practice tend to discourage many patients from the necessary permanent continuance of dieting. The central thesis propounded in 1922 has not been fully accepted by any later writer, namely, that salt is a specific aggravating factor in a specific disease, essential hypertension, the progressiveness of which can be controlled by eliminating this factor. This is comparable to the control of progressiveness in diabetes by control of the sugar.

This simple theory is strengthened by accumulating evidence. First, former contradictions have been withdrawn (A. Grollman *et al., J. Am. Med. Assn.* **129,** *533 (1945)*) and there is confirmation from animal experiments (F. M. Allen and O. M. Cope, *J. Urology* **47,** *751 (1942)*; R. Lenel, L. N. Katz, and S. Rodbard, *Am. J. Physiol.* **152,** *557 (1948)*) showing that salt ingestion raises blood pressure in the normal and hypertensive organism.

Secondly, H. Selye's demonstration (*J. Am. Vet. Med. Assn.* **103,** *140 (1943)*; Selye and E. I. Pentz, *Canad. Med. Assn. J.* **49,** *264 (1943)*; Selye and H. Stone, *J. Urology* **56,** *399 (1946)*) that chronic hypertension

may be produced by adrenal or pituitary hormones, particularly in association with salt, furnishes strong evidence for salt not merely as a secondary aggravating influence but as an actual etiologic factor.

Thirdly, the profound vascular atony of shock is combated specifically by administration of sodium salts (F. M. Allen, *Arch. Surgery* **38**, *155 (1939); Am. J. Surgery* **61**, *79 (1943);* **62**, *80 (1943)*). Numerous confirmations can be cited of this work, together with the fact (E. Mylon, M. C. Winternitz, and G. J. de Sütö-Nagy, *Am. J. Physiol.* **139**, *313 (1943)*) that dialyzed plasma is valueless in shock.

Fourthly, clinical observations, including W. Kempner's statistics (*North Carolina Med. J.* **6**, *61, 117 (1945); Am. J. Med.* **4**, *545 (1948)*) have convinced most physicians of the benefit of restricting salt in hypertension, as previously mentioned.

That vascular changes should sometimes be so advanced as to resist a functional treatment is a rational expectation. The sclerosis may in fact be such that even vasomotor crippling by drastic sympathectomy fails to reduce blood pressure.

The lack of special virtues in rice or of special harm in protein or condiments is indicated by a comparison. Kempner and other advocates of the "rice diet" are now where we were in 1922, with a four year experience, the same benefits, and the identical proportion of refractory cases, namely 30 per cent. The question of mere symptomatic relief versus specific arrest of a disease can be settled only by adequate clinical experience. To my knowledge no one has yet claimed that benefit obtained with a nutritionally adequate, really low salt diet has ever disappeared on continued diet.

Most physicians discussing strictly low salt diets lack sufficient duration of experience for judging long-term results. It was once hoped that our experience now amounting to several thousand cases during thirty years would furnish adequate statistical evidence of long-term effectiveness. However, we were unable to continue the system of complete data and follow-ups which is indispensable for valid statistics.

Some information is available nevertheless. Positiveness can be added to the statement which was hazarded from 1922 to 1925, that many initially refractory cases show arrest or gradual improvement during years of fidelity to a really low salt diet, in a manner not yet duplicated by surgery, rest, drugs, or any other treatment.

It also appears significant, as previously reported, that this control of essential hypertension is not equaled in nephritic hypertension. Statistical proof that both resistant and responsive patients live far longer than the accepted average, might still be questioned, just as I question the excellently prepared statistics of surgeons, and never endorse sympathectomy for any patient.

Cases which appear initially alike are known to vary widely in their course. Also, in honesty, strikingly better results in our series of private patients than in some hospital's series of charity patients could not constitute scientific proof of the low salt theory. Our patients ordinarily die from coronary or cerebral consequences of arteriosclerosis which antedated treatment. There are deaths occurring soon after beginning treatment, and there can seldom be positive proof that the early or late time in any individual is determined by treatment.

However, about 50 per cent of cases of essential hypertension ordinarily terminate in congestive heart failure. Retinitis, angina, and uremia are notoriously frequent. Stronger than any statistics is the sweeping declaration that in our experience these are prevented by a continuous low salt diet. Since salt is merely the principal, not the sole influential factor, rare exceptions may prove the rule, but we have not thus far encountered them.

The practical manual previously referred to (published in 1925, and still obtainable) included numerous instructions, recipes, and also a set of sample menus (*pp. 188–198*) rang-

ing in energy value from a reducing ration of 776 calories to an overnutrition ration of 3322 calories, and from a salt-free protein-free diet through increasing amounts of protein to 80 g. daily. It also emphasized the importance of skill and imagination in cooking, to obviate the tastelessness which is now a source of complaint from those who evidently lack these qualities. One example of a liberal diet is the following.

MENU

Salt-free, Protein 70 g., Calories 2781

	NaCl	Protein	Fat	C.H.
Breakfast:				
Coddled egg (1)	.0900	7.0	5.5	...
Hominy 20 g.	.0001	2.4	1.6	15.8
Popover (1) (by dietitian)	.0501	4.8	4.6	19.8
Grapefruit 100 g.	.0045	...	...	5.0
Sugar 20 g.	....	...	...	20.0
Cream 50 g.	.0680	1.5	10.0	2.5
Butter 10 g.	.0130	...	8.5	...
Noon Meal:				
Roast Pork 80 g.	.0995	15.5	20.2	...
Apple sauce: Apple 100 g.	.0080	1.0	...	20.0
Apple sauce: Sugar 15 g.	....	...	...	15.0
Apple sauce: Water ½ cup	....	...	...	...
String beans 100 g.	.0400	2.0	...	7.0
Lettuce 30 g.	.0375	0.3	...	0.9
Watercress 20 g.	.0200	0.2	...	0.8
Sponge cake (by dietitian)	.0996	8.0	4.3	43.8
Bread 30 g. (salt-free)	.0378	3.0	0.3	16.5
Sugar 10 g.	....	...	...	10.0
Cream 30 g.	.0408	0.9	6.0	1.5
Butter 10 g.	.0130	...	8.5	...
Dinner:				
Tenderloin Steak (broiled) 60 g.	.0963	15.0	12.4	...
Mushrooms 50 g. (no food value)	.0180	...	...	...
Butter 20 g.	.0260	...	17.0	...
French Fried: Potatoes 100 g. (raw weight)	.0650	2.0	...	15.0
French Fried: Oil 50 g.	....	...	50.0	...
Stuffed Tomato Salad: Onion 5 g.	.0018	...	...	0.2
Stuffed Tomato Salad: Green pepper 5 g.	.0011	...	...	0.5
Stuffed Tomato Salad: Lettuce 50 g.	.0620	0.5	...	1.5
Stuffed Tomato Salad: Mayonnaise 10 g.	.0024	0.2	9.0	...
Stuffed Tomato Salad: Tomato pulp 80 g.	.0456	0.8	...	3.2
Canteloupe 100 g.	.0700	1.0	...	10.0
Parker House rolls 30 g. (by dietitian)	.0378	3.0	0.3	16.5
Sugar 10 g.	....	...	...	10.0
Cream 30 g.	.0408	0.9	6.0	1.5
Butter 10 g.	.0130	...	8.5	...
Total	1.1017	70.0	172.6	237.0

The following reasons may be given for the superiority of these original low salt diets over many of those recently proposed.

1. Except for brief emergencies, the predominant requisite of a saltless diet must generally be its suitability to be followed faithfully throughout a patient's life. This purpose is served by offering an unlimited variety of natural foods, without insistence on artificial or dialyzed preparations such as may be used for temporary or experimental regimens in institutions, and with ample vitamins and other accessory factors not requiring artificial supplements.

2. The sodium chloride intake, which may appear unduly high, nevertheless will usually conform to the stipulated standard, namely, not more than 0.5 g. of urinary sodium chloride daily. There is no proof that the blood pressure is affected by the fraction of salt which is lost through the bowel or skin. There was apparent clinical proof that the restriction to 0.5 g. of sodium chloride in the urine affects blood pressure as favorably as stricter standards, even down to a chloride-free urine (cf. Manual, *p. 80*). Actually, many less severe cases can tolerate up to 1.0 g. in the daily urine. Also, a wide experience indicates that this type of diet can be followed through all the variations of weather and climate prevailing in the United States, with only very rare liability to the salt deficiency dangers which have been described with some more stringent diets or the addition of mercurial diuretics.

3. For some conditions, especially severe congestive heart failure or other stubborn edemas, the need for a lower salt intake is recognized. In other cases the urinary sodium chloride will persistently exceed the prescribed standard. The salt content of the water supply, which has recently been noticed (S. L. Cole, *J. Am. Med. Assn.* **140,** *19 (1949)*) is generally less important than another source which has not been duly recognized, namely, the variable salt content of vegetables, especially the high content of those grown in salty soil. Any of these problems can be solved by merely boiling the meats and vegetables through one to three changes of distilled water. Any loss of flavor can be compensated by proper use of con-

diments. The sodium chloride content can thus be reduced to a fraction of the tabulated figure without resort to artificial diet plans.

It is particularly amazing that the authorities who now enthuse over the benefits in treatment of congestive heart failure to which they were so long blind, generally remain blind to this value in *preventing* congestive failure (F. M. Allen, *J. Am. Med. Assn.* **133,** *20 (1947)*). This is our challenge to those who talk about the unchangeable evolution of essential hypertension, who lack any real experience with really low salt diets to back their opinions. Several thousand physicians using our original diet, Kempner's rice diet, or other really low salt diets are aware that established retinitis or angina may not be curable, but they can testify whether they have seen any exception to our experience that these complications or sequels do not arise on continuous proved salt exclusion.

These clinical studies support our contention that a specific disease, essential hypertension, which in its ordinary nature is characterized by rapid or slow progressiveness, is arrested or reversed by elimination of a specific aggravating factor, salt. Beyond that they should stimulate a longer and slower demonstration which we once hoped to furnish, namely, prevention of arteriosclerosis by treatment begun in the incipiency of hypertension.

There is not space in this brief review for consideration of the pathology, beyond a reminder that essential hypertension typically begins as a functional disorder, temporarily controllable by simple rest. It is rational that sodium salts, with their powerful and specific influence upon vascular tone and permeability, should be a specific and dominant factor in the vasoconstriction of this disease.

The lesson for nutrition is plain. The components of salt, sodium and chlorine, are minerals essential in nutrition. Salt is a valued condiment and is necessary in increased amounts under special conditions, for example, profuse perspiration. The comfortable endurance of all ordinary weather conditions by thousands of patients on rigid salt-poor diets shows that this need has been exaggerated.

Particularly, however, there is widespread abuse of salt for preservation and for satisfying artificially perverted tastes. While there should be special care in salt restriction in families with inherited hypertensive tendencies, education against the prevalent abuse is the preeminent public health requirement for reducing the incidence of hypertension, which is now among the principal causes of death in the civilized world.

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TREATMENT OF PERNICIOUS ANEMIA

The comparative therapeutic effectiveness of the pteroylglutamates and vitamin B_{12} on the hematologic and neural abnormalities of pernicious anemia has been reviewed (*Nutrition Reviews* **6,** *245, 291 (1948)*; **7,** *255 (1949)*). These observations have demonstrated that pteroylglutamic acid does not completely replace potent extracts of liver in the treatment of this disease. Hematologic relapse and the appearance or progression of clinical evidences of combined system disease occur frequently despite continuation of therapy. Clinical experience with vitamin B_{12} indicates that this substance does not have the same therapeutic limitations and may be as effective in treatment as is liver extract.

Changes in the mucosa of the tongue occur as a frequent manifestation of pernicious anemia. The abnormality of atrophy and inflammation of the lingual papillae is not a life-threatening sign of the disease. Yet it

is a readily recognizable manifestation and permits another means of evaluation of therapy. Treatment with extracts of liver or of hog stomach uniformly bring about prompt regression of the lesion.

Although J. F. Wilkinson (*Brit. Med. J.* **1,** *771 (1948)*) reported that soreness of the tongue disappears during treatment of pernicious anemia with pteroylglutamic acid, other workers have found that in certain patients so treated there is failure of glossitis to abate or that the lesion recurs after initial temporary improvement (J. F. Ross, H. Belding, and B. L. Paegel, *Blood* **3,** *68 (1948)*; B. E. Hall and C. H. Watkins, *J. Lab. Clin. Med.* **32,** *622 (1947)*; P. F. Wagley, *New Engl. J. Med.* **238,** *11 (1948)*).

J. F. Schieve and R. W. Rundles (*J. Lab. Clin. Med.* **34,** *439 (1949)*) record observations relative to the response of the lingual manifestations of pernicious anemia to treatment with pteroylglutamic acid and vitamin B_{12}. The patients treated exhibited typical clinical manifestations of the disease with histamine refractory gastric achlorhydria. When pteroylglutamic acid was administered several types of response of the lingual papillae were observed.

In 2 patients given an oral dose of 75 mg. of pteroylglutamic acid daily there was rapid regression of papillary atrophy and inflammation with complete restoration of normal lingual mucosa which was maintained. However, this agent in relatively large dosage failed to produce an adequate response in 4 patients. There was either complete lack of regeneration of papillae or recurrence of atrophy after apparent initial improvement. Liver extract was subsequently administered to these patients and was effective in correcting the lingual defect. In these patients, also, pteroylglutamic acid was without effect upon the hematologic and neural manifestations of the disease.

Although there was satisfactory response by other means of assessment in 2 additional patients, relapses in lingual lesions occurred after three months of continued treatment. When 25 micrograms of vitamin B_{12} were administered intramuscularly to one of these patients after failure of pteroylglutamic acid, soreness of the tongue promptly subsided. In 5 patients, vitamin B_{12} in daily intramuscular doses of 1 microgram or after a single injection of 10 micrograms effected complete regeneration of lingual papillae in the relatively short interval of from five to seven days. No failure of treatment with vitamin B_{12} was recorded.

The observations which compare the therapeutic effectiveness of pteroylglutamates and vitamin B_{12} against the lingual atrophy and inflammation of pernicious anemia, corroborate the evidence obtained by other means of appraisal. Vitamin B_{12} seems entirely adequate in replacing potent liver extracts as determined by hematologic, neurologic, and lingual response to therapy. On the other hand, pteroylglutamic acid, although frequently producing measurable or effective response, is seriously limited in therapeutic effect. It cannot be considered a satisfactory mode of therapy for any of the manifestations of the disease. The experience of Schieve and Rundles indicates that failure of lingual response to pteroylglutamate is usually associated with failure of the drug to elicit hematologic or neurologic control of symptoms.

There is no constant relation between the occurrence of hematologic, neurologic, and lingual manifestations of pernicious anemia. Any one of these three types of manifestation may occur alone or in combination with the others. The concept is held by some that pernicious anemia may be the result of a deficiency of a number of factors and that the varied manifestations are results of different combinations of deficiencies. There seemed some support for this thesis in earlier statements that pteroylglutamic acid was effective in treating the red blood cell defect but relatively ineffective against neural manifestations. Information presently available indicates that this substance is equally

ineffective against each of the three types of clinical manifestations. In contrast, vitamin B_{12} provides prompt and adequate therapy for all abnormalities which accompany the disease. This would seem to indicate a single type of metabolic defect or deficiency.

DIET AND REPAIR OF EXPERIMENTAL HEPATIC CIRRHOSIS

Experimental hepatic cirrhosis has been shown to undergo a reversal when administration of para-dimethylaminoazobenzene or of carbon tetrachloride is discontinued (J. W. Orr, *J. Path. Bact.* **50,** *393* (*1940*); G. R. Cameron and W. A. E. Karunaratne, *Ibid.* **42,** *1* (*1936*)).

T. G. Morrione (*J. Exp. Med.* **85,** *217* (*1947*)) compared the results of a chemical quantitative method for determining the content of collagen in cirrhotic livers produced by the above hepatotoxic agents with parallel histologic observations. From the results of this comparison he concluded that there was good correlation between the two methods for evaluation of collagen content in livers.

T. G. Morrione (*Am. J. Path.* **25,** *273* (*1949*)) has continued his studies on experimentally induced cirrhosis with the object of determining whether certain dietary constituents fed after the discontinuation of carbon tetrachloride poisoning had any effect on the rate of recovery from cirrhosis. Hepatic cirrhosis was produced in 234 out of 252 young male albino rats by exposure to carbon tetrachloride vapor in a closed vessel for a period of about twenty minutes on alternate days for a total of thirty-five days. Groups of rats were killed at five-day intervals and collagen determined on 2 to 3 g. samples of their livers to determine the rate of collagen deposition. On the thirty-sixth day, remaining rats selected at random were divided into seven groups.

The first group was fed a normal diet. Five groups of 20 rats each were offered special diets: low protein; low protein supplemented with 500 mg. of methionine, 125 mg. of choline, and 312.5 mg. of l-cystine per 100 g. of diet; high protein; low protein, high fat; and high carbohydrate, low fat. Ligation of the portal vein was performed on the rats in the seventh group. Six normal control rats were killed to determine the normal value of hepatic collagen, and ligation of the portal vein was performed on 12 additional normal rats.

Collagen content of livers due to cirrhosis of carbon tetrachloride origin increased from 0.16 to 0.54 per cent during the thirty-five day period of treatment. Reversal of cirrhosis occurred when rats were maintained on a diet of dog chow pellets after the carbon tetrachloride treatment was discontinued. The collagen content decreased from 0.51 per cent on the third day after last exposure to 0.32 per cent on the thirty-eighth day. The incompleteness of the repair process was further demonstrated by microscopic evidence of persisting cirrhosis.

The average collagen content of the three groups of rats receiving the low protein, the high protein, and the high carbohydrate low fat diets decreased at about the same rate as that of the animals on the dog chow diet. The low protein diet supplemented with methionine, choline, and l-cystine permitted a slightly more rapid withdrawal of hepatic collagen. On the other hand, the low protein high fat diet permitted essentially no withdrawal of collagen from the cirrhotic livers. Likewise, the collagen content remained high when the portal vein was ligated immediately after discontinuation of carbon tetrachloride exposure unless adhesions developed. There was a suggestion that the formation of adhesions between the liver and adjacent structures in rats, where the portal vein had been ligated, resulted in a greater ability to recover from cirrhosis. Ligation of the portal vein in

normal rats resulted in a relative and an absolute increase in the collagen content of the liver.

These studies seem to be preliminary in nature but merit continued investigation as a method of studying mechanisms which govern ability of the body to remove collagen from the liver accumulated during the administration of hepatotoxic agents. Although these investigations appear to have no immediate application in human disease control or alleviation, they offer promise of providing data which may be of importance in understanding certain phases of liver disease in which cirrhosis is one of the complicating developments.

EFFECTS OF THIAMINE RESTRICTION AND SUPPLEMENTATION IN PSYCHIATRIC PATIENTS

W. Stephenson, C. Penton, and V. Korenchevsky (*Brit. Med. J.* **2,** *839* (*1941*)) reported positive effects of supplementation of a hospital diet with thiamine and ascorbic acid. The effects were noted both with reference to the physical and mental health of the patients who were inmates of an English mental institution. The authors stated: "By treating aged people with vitamins, it was possible to prevent or improve, in some cases to a striking degree, certain of those senile features which could be considered as pathological, because they appeared prematurely or in an extreme degree (e.g., muscular, cardiovascular and mental deterioration) or which did not seem to be inevitable in normal physiological senility (e.g., dementia, insomnia, skin rashes, itching, constipation)." The implications were far reaching. Obviously, the claims needed rechecking and a more thorough documentation of the findings was desirable.

The British report stimulated setting up of a project at the Elgin State Hospital the original purpose of which was to compare over a period of years the effects of liberal allowances versus moderately restricted allowances of B-complex vitamins on the psychologic and physical status of mental patients while other dietary factors and the environment were kept relatively constant. The study was conducted by members of the staff of the Elgin State Hospital under the supervision and guidance of the Committee on the Nutritional Aspects of Aging (Food and Nutrition Board, National Research Council). A general report of the study has been published (M. K. Horwitt, E. Liebert, O. Kreisler, and P. Wittman, *National Research Council Bull.* **116** (*1948*)). The psychiatric aspects were summarized separately (Kreisler, Liebert, and Horwitt, *Am. J. Psychiat.* **105,** *107* (*1948*)).

The study was to cover all of the major aspects of human biology and medicine, and included biochemical, clinical, neurologic, and psychologic observations. The original design provided for three groups: (1) Group A was to be maintained on a standard diet providing daily about 2200 calories, 400 micrograms of thiamine, and 800 micrograms of riboflavin (188 micrograms of thiamine and 367 micrograms of riboflavin per 1000 calories as compared with the National Research Council's recommended allowances (1948) of 500 and 750 micrograms, respectively). On page 5 of the monograph the values are erroneously given as milligrams. (2) Group B was to receive the same standardized diets, consisting of three rotated but relatively monotonous menus, supplemented with 6 mg. of brewers' yeast extract. The supplement provided not less than 3600 micrograms of thiamine and 2300 micrograms of riboflavin per day. The subjects of group A received placebos resembling the yeast extract. (3) Group C was allowed free access to the general hospital diet which provided approximately 2500 calories, 1000 micrograms of thiamine, and 1500 micro-

grams of riboflavin per day. The first two groups consisted of 5 younger men (mean 31 years of age in both groups A and B) and 7 older men (mean 69 years of age in group A, 64 years of age in group B). Group C consisted of 8 younger and 7 older men (mean 27 years and 72 years). The younger subjects were diagnosed as suffering from schizophrenia. The older men exhibited simple senile deterioration or various types of psychosis.

After two years of maintenance on a dietary regimen which provided the control (group B) with ten times as much thiamine as the experimental (moderately restricted, A group), no incontrovertible differentiation in the status and the trend of changes of the two groups was obtained. However, some of the investigators suspected mild clinical abnormalities and, in particular, differences in the response to imposed metabolic loads (glucose plus mild exercise) were noted between the subjects on the restricted and control diet.

It is likely that the physical and mental status would be changing as a result of progressing mental illness and/or the normal process of aging. Consequently, in evaluating the statistical significance of the changes in the moderately restricted group, the mean difference between the pre-experimental status of group A and its status after two years, must be corrected with reference to the mean difference between the two periods observed in group B. Rigorous statistical analysis was applied only to a small fraction of the psychologic data. No significant effect of the experimental (restricted) diet could be demonstrated for measured mental performance. The only significant difference in the psychologic area was obtained for the Elgin Test Reaction rating scale. The clinical observations indicated that the restricted subjects were more lethargic and had labile emotional reactions. The resistance of intellective functions to deterioration in the face of changing attitude and temperament is in harmony with the data obtained at the University of Minnesota (H. Guetzkow and J. Brozek, *Am. J. Psychol.* **59,** *358 (1946)*). No impairment of superficial sensibility developed and neither loss of muscle power nor ataxia could be demonstrated in any of the A group members.

During the third year, group A was continued on the moderately restricted regimen while the thiamine intake of group B was drastically reduced (92 micrograms per 1000 calories). The reduction was accomplished by withdrawing the yeast extract supplement, substituting rice for potatoes and spaghetti, and serving biscuits instead of loaf bread. The diet provided 340 micrograms of riboflavin. Marked abnormalities, both biochemical and clinical, developed in the severely restricted subjects within a few months. Within three weeks the twenty-four hour excretion of thiamine decreased from about 2000 micrograms to 50 micrograms and reached practically a zero level after three months on the severely restricted diet. Biochemically, a thiamine deficiency involves a disturbed carbohydrate metabolism. It is of interest, therefore, that the basal levels of lactic and pyruvic acid proved to be of little, if any, value in estimating the state of nutritional deficiency. On the other hand, there was a precipitous rise in Horwitt's "index of carbohydrate metabolism," obtained by relating glucose, lactic acid, and pyruvic acid levels of the blood after a combined glucose ingestion and exercise test. Objective evidence of polyneuritis was observed with constancy when this index exceeded 15 and in such instances the index dropped precipitously within a few days after restorative treatment with thiamine was instituted.

Irritability increased, complaints of paresthesias of varied nature, such as tingling, burning sensations, and "pins-and-needles" were heard frequently, disturbances of superficial sensitivity were noted, and position sense and the acuteness of vibratory sense were impaired. Some of the subjects had difficulties in arising from the squatting

position and in climbing stairs. In all subjects of the severely restricted group there was a decrease or loss of deep reflex responses. In the electrocardiographic tracings the T waves in leads I and II decreased and disappeared in lead III. Mental efficiency was considered to be lowered in a number of patients but large individual differences were noted. Two of the younger subjects and one of the older subjects did not show evidence of a lowered mental efficiency even up to the time when the clinical signs of deficiency made it mandatory to terminate the dietary restriction. On supplementation, the recovery was dramatic in some functions, such as paresthesias and general display of energy. Among the slowest functions to recover was the Achilles tendon reflex.

The project was originally designed to provide additional information on minimal requirements for thiamine and riboflavin, with special reference to the relative need of younger versus older men. The second object was to study the influence of low and high intakes of these vitamins on the symptoms of the mentally ill. The intake of approximately 200 micrograms of thiamine per 1000 calories in inactive men whose diet provides daily about 2000 calories did not lead to definite or marked alterations of the clinical state, but it was judged to be below the minimal requirement. No evidence concerning minimal riboflavin intake was obtained.

Contrary to an earlier assumption, no conclusive evidence was provided that thiamine requirements are larger in the aged than in younger adults. Severe restriction of thiamine intake definitely intensified many of the neurologic and psychiatric symptoms of neuropsychiatric patients. On the other hand, there were no indications that liberal provision of thiamine (and other components of the vitamin B-complex) would be of therapeutic value in the treatment of mental disease.

ELIMINATION OF TRACER CARBON FROM TISSUES OF RATS

The amount of radioactive carbon retained in the tissues of growing and mature animals is a problem of considerable importance in the evaluation of the potential health hazards of this long-lived isotope (approximately 5100 years).

W. D. Armstrong, J. Schubert, and A. Lindenbaum (*Proc. Soc. Exp. Biol. Med.* **68,** *233 (1948)*) report results of an investigation in which radioactive carbon was administered to unfasted mature rats either by intraperitoneal injection of labeled sodium carbonate or by direct implantation of labeled calcium carbonate into the peritoneal cavity. Within six hours after injection of sodium carbonate, about 95 per cent of the total injected dose of radioactive carbon had been expired in the carbon dioxide. When these rats died at the end of a hundred and sixty hours, only 0.45 per cent of the initial isotope dosage was still present in the entire carcass.

Administration of calcium carbonate prolonged the elimination of labeled carbon; the peak of radiocarbon in the expired air came on the seventh and eighth days. In one rat maintained after implantation for twenty-two days, 0.38 per cent of the radioactivity in the original dose was still present in the carcass; in a second rat given labeled calcium carbonate, 1.0 per cent of the original dose was present at the end of six days. Of the total dose administered in the latter animal, 0.022 per cent was present in the long bones, 0.068 per cent in the short bones, 0.00037 per cent in the molar teeth, 0.00130 per cent in the incisor teeth, 0.0112 per cent in whole blood, 0.365 per cent in the liver, 0.0629 per cent in muscle protein, 0.0305 per cent in body fat, 0.0253 per cent in the proteins of

the digestive tract, 0.00150 per cent in the brain and spinal cord, and 0.130 per cent in the pelt.

The greatest incorporation of radioactive carbon in bones and teeth took place in the inorganic carbonate fraction. The radioactive content of this fraction accounted for almost 90 per cent of the total activity in the skeleton. The specific activity of the inorganic carbonate in the enamel and dentin of the developing incisor was as high as that of the inorganic carbonate in the femur.

J. Schubert and W. D. Armstrong (*J. Biol. Chem.* **177,** *521* (*1949*)) gave 8 mature and 6 growing rats intraperitoneal injections of sodium carbonate labeled with radioactive carbon in sufficient amounts to represent 0.13 to 0.25 millicurie per rat, to study the change in radioactive carbon concentration of the soft and calcified tissues and derived components with time. The rats were killed in pairs at different times from eight to thirty days after the last of three equal injections that were given at two hour intervals. By the eighth day after administration of labeled sodium carbonate, the specific radioactivity of all tissues, with the exception of the serum proteins in the growing rats, was much higher than for the mature animals. The amounts of radioactivity and the rate of change in the serum proteins were approximately the same for the two groups of rats.

The higher concentration of radiocarbon in the tissues of growing rats would appear to be a result of the reconstitution of existing tissues plus the growth of new tissue incorporating radioactive carbon from the extracellular fluids. In mature rats, incorporation of radiocarbon would occur only through the process of reconstitution of existing tissues. Though the specific radioactivity of individual tissues and organs was higher in growing rats, the over-all retention of radiocarbon was appreciably greater in mature rats due to their greater total body weight.

In young rats, the total retained radioactivity decreased from 0.55 per cent of the original dose on the eighth day to 0.22 per cent on the twenty-second day; in mature rats, the decrease for the same experimental period was from 0.96 per cent of the original dose to 0.50 per cent. The radioactive carbon content of the skeleton decreased continuously through the period of observation.

The biologic half-life of the radioactive carbon (the length of time necessary for the turnover of isotope within a tissue to cause a 50 per cent reduction in the original concentration of the isotope) in whole bone and in inorganic carbonate in bone, in the period between the twentieth and thirtieth days after injection, was estimated to be about twelve to fifteen days in growing rats and thirty to forty days in mature rats. By the thirtieth day in mature animals, the residual radioactivity in the soft tissues was still decreasing, but at a much reduced rate. In the growing rats, the radioactive carbon concentration in the soft tissues was decreasing at a much more rapid rate at the end of the experimental period, indicating the more rapid metabolic processes of the growing animal.

The specific activity of the growing incisor teeth of mature rats was at least five times that of the nongrowing molar teeth. In young rats, where calcification was still taking place in the molars, there was little difference in the amounts of radiocarbon incorporated into the incisor and molar enamel and dentin.

These data indicate the rapidity with which the major portion of injected doses of sodium carbonate and calcium carbonate labeled with radioactive carbon is metabolized and excreted in the expired carbon dioxide. In addition, those small amounts left after the first few hours of rapid excretion are gradually metabolized and expired. In this way, readily metabolized compounds of radiocarbon would appear to be relatively innocuous despite the extremely long half-life of this isotope. The amounts of radiocarbon

that are incorporated into the tissues and their components during the period of rapid metabolism and expiration are gradually lost, thus preventing continued accumulation of dangerous levels due to repeated exposure.

Many additional studies need to be conducted before full knowledge is known of this isotope and its effects. One interesting point of this type was the demonstration that brain protein in young animals had no significant turnover of radioactive carbon following its incorporation into that tissue during growth and development.

AMINO ACIDS AS THE SOLE DIETARY NITROGEN SOURCE IN DOGS

The adequacy of amino acid mixtures in substituting for protein in the nutrition of the dog has been investigated by a number of different groups. S. C. Madden and co-workers (*J. Exp. Med.* **77,** *277 (1943)*) and W. C. Rose and E. E. Rice (*Science* **90,** *186 (1939)*) reported that the ten amino acids found by Rose to be essential in the diet of the rat would satisfy the requirements of the dog for plasma protein production, weight maintenance, and nitrogen balance. However, L. L. Miller, F. S. Robscheit-Robbins, and G. H. Whipple (*J. Exp. Med.* **85,** *267 (1947)*) were unable to maintain weight or blood proteins in their dogs fed a mixture of purified amino acids.

Examination of the food consumption data of the latter group led R. H. Silber, E. E. Howe, C. C. Porter, and C. W. Mushett (*J. Nutrition* **37,** *429 (1949)*) to believe that the loss of weight which Miller and co-workers observed in dogs on amino acid mixtures resulted from a loss of appetite with a consequent inadequate caloric intake, rather than from a nutritional inadequacy of the amino acid mixture *per se*. They have consequently carried out experiments with adult dogs and with puppies to determine the adequacy of amino acid mixtures as the sole source of dietary nitrogen. Their mixtures of amino acids were obtained by complete acid hydrolysis of casein, followed by purification of groups of amino acids and recombination to give diets containing known amounts of the essential amino acids, with some nonessential amino acids as well. Hydrolysis of the casein was complete, as evidenced by a negative biuret reaction and failure to obtain an increase in alpha-amino nitrogen on further hydrolysis.

This mixture was fed with a protein-free ration to adult dogs for forty days, then subsequently administered intravenously for an additional twenty-four days. During this period, the dogs maintained their initial weights and the protein content of the blood showed no decrease. Over the same time interval, dogs fed casein with the basal ration behaved in a manner similar to those given the amino acid mixture; controls given the protein-free ration alone lost weight, showed a lowered plasma protein, and developed a normocytic anemia.

Subsequently, 6 Beagle puppies, 12 weeks of age, were divided into groups of 2 each, which were fed the amino acid mixture, casein, or no supplement together with the protein-free ration. The food consumption of the casein-fed group was limited to that of the amino acid-fed group. Both groups gained weight at essentially the same rate (0.40 kg. per week for the amino acid-fed group, 0.43 kg. per week for the casein-fed group) over the twelve weeks of the experiment. The group given only the control, protein-free ration lost weight during the first month of the experiment. They were then transferred to the amino acid ration, on which they subsequently showed steady weight gains.

When first given the amino acid ration, the pups ate it rapidly and vomited shortly thereafter. Within several days, however,

they learned to consume it in small portions which they retained in amounts sufficient to permit growth. It is interesting to note that if the pups had not learned to eat the diet in small portions, it would have been considered inadequate in the usual nutritional sense.

Neither the adult dogs nor the pups showed pathologic symptoms from ingestion of the amino acid rations. Both showed a slightly higher albumin to globulin ratio in the plasma proteins than did the casein-fed controls, the significance of which is not known.

Over the period of these experiments, therefore, a peptide-free mixture of amino acids was the nutritional equivalent of intact protein, and no evidences of toxicity were observed. The only limitations of the nutritional utility of the mixture were those imposed by its tendency to produce nausea, which resulted in smaller food consumption, with consequent limited weight gains, as compared with what might have been obtained in pups allowed to consume, ad libitum, a ration containing casein.

Although the authors' conclusion that peptides are not essential for growth or maintenance of dogs is certainly valid under the conditions of this experiment, the limited time span of the experiments, and the decreased growth rate of the pups over this time make further experimentation necessary to prove the conclusion justified when applied to the entire life span of the dog.

RATE OF DIGESTION OF HEAT-PROCESSED PROTEINS AND THEIR BIOLOGIC VALUE

That the extent of heat processing of many protein foods affects their biologic value has been recognized for some time. Soy protein is particularly illustrative of a case in which heat processing greatly increases biologic value, but other legume proteins have also been reported to behave similarly. In these cases the essential amino acid, methionine, appears to be made "available," since addition of methionine to the raw soy protein largely corrects the discrepancy of the biologic value of the raw and processed proteins.

Excessive heating of many protein foods, on the other hand, causes a decrease in biologic value. In these cases, addition of the amino acid, lysine, appears to correct the deficiency produced. Since the methionine or lysine contents of these altered proteins have not been changed, various theories have been offered to explain the change in biologic value.

The above facts have been thoroughly reviewed and documented by D. Melnick and B. L. Oser (*Food Technology* **3,** *57 (1949)*), and these investigators have offered a theory with considerable experimental support for the differences in the biologic activities of heat-processed proteins. They cite various reports (see also *Nutrition Reviews* **5,** *316 (1947)*) which indicate that the proper amino acid mixture must be presented to the tissues at a given time for optimal utilization in tissue protein synthesis. A delay in the supply of an essential amino acid might thus cause a relative waste of the other amino acids, a situation clearly evident in the exaggerated situation of a complete lack of an essential amino acid. Thus Melnick and Oser suggest that the difference in biologic values of a protein subjected to various degrees of heat depends on the rate of liberation of its constituent amino acids during enzymatic digestion.

In such a way, they believe, differences in the biologic value of the same protein can be related to the rate of enzymatic digestion of the protein, and independent of the "coefficient of digestibility," the latter being an over-all percentage of nitrogen absorbed.

Experimentally, these investigators have proceeded to develop an *in vitro* technic to measure the rate of liberation of amino acids from proteins during digestion by commercial preparations of "Pancreatin," and later to measure the rate of liberation of methionine and lysine which seemed more specifically concerned. The "formol titration" was adopted to measure the liberation of amino acids from the test material, and these measurements were commonly made after one, two, and five days of Pancreatin digestion of a toluene-preserved sample. The initial formol value was determined on a mixture of the protein and heat-inactivated Pancreatin, and the formol value for complete hydrolysis by refluxing the mixture with 8 normal sulfuric acid for twenty-four hours. With these data the "per cent digestibility" *in vitro* at different times could be calculated.

The accuracy and suitability of the method was tested in several ways. Thus the formol titration was studied with several amino acids to determine the end-point pH which best corresponded to the nitrogen content of the amino acid. A pH of 9.5 was chosen as the end point, and this selection was justified by finding close agreement between the nitrogen analyses of a variety of food protein hydrolysates as determined by the Kjeldahl and formol titration methods. The presence of proteolytic enzymes in the protein tested for *in vitro* digestibility interferes with the test, and such enzymes must first be inactivated. A large amount of fat in the protein sample tested also interferes, hence samples were defatted if the fat content exceeded half the protein content. With adequate control, the digestibility test was found to be accurate and reproducible.

The method was first applied to soy flour which is known to undergo, upon autoclaving, a marked increase in biologic protein value though no change in "coefficient of digestibility" or amino acid composition. Heating conditions known to increase the biologic value caused an increase in the *in vitro* digestibility. Thus 8, 9, and 11 per cent of the amino acids were liberated from unheated soy flour after one, two, and five days, respectively, while after autoclaving the flour for thirty minutes at 10 pounds pressure, the corresponding amino acid liberations were increased to 14, 23, and 29 per cent. Among other protein materials tested, a marked increase in amino acid release by Pancreatin occurred when raw whole egg was hard boiled, and when the proteins of whole beef blood were heat coagulated.

Other studies on soy products compared the *in vitro* digestibility tests with biologic values (rat assay) and the biologic "coefficient of digestibility" on the same materials. The biologic values of extracted and toasted soy meal, expeller soy meal, and raw soy meal decreased in the order listed, and the same order was found in the enzymatic digestibility test. In another series of soy meals the same parallelism of biologic value and *in vitro* digestibility was found, the biologic values for heat-processed and raw soy meal being 71 and 53 per cent, respectively, and the digestibilities 37 and 22 per cent, respectively. The biologic "coefficient of digestibility" decreased only from 84 to 81 per cent.

The investigators interpret these results to mean that the heat processing does not make any more total methionine (the limiting amino acid in raw soy meal) available, yet the percentage of absorbed nitrogen retained for tissue synthesis is much greater, and that this is due to liberation of methionine at a time when it most favorably supplements the other amino acids. The possibility was considered that the small decrease in the "coefficient of digestibility" of raw soy meal might be due largely to lack of methionine absorption, thus providing an alternative explanation of the results. Experiments in this direction, however, failed to indicate any difference in fecal excretion of methionine on the different types of soy meal, and the results supported earlier work which indicated that the change in methio-

nine metabolism involved greater retention of absorbed methionine from the heat-processed products rather than a change in absorption.

The theory of Melnick and Oser would of course be strengthened by direct demonstration that the methionine of heat-processed soy meal is released sooner in the course of enzymatic digestion than in the case of raw soy meal. Studies in this direction were undertaken by the investigators. Toward this end, the leucine, lysine, and methionine contents of trichloracetic acid filtrates prepared from the enzymatic digests of raw and heat-processed meals were determined. In both cases methionine was liberated more slowly than lysine, but only 36 per cent of methionine was found in the five day hydrolysate of raw soy meal as compared with 73 per cent in the hydrolysate of processed meal. Hence the methionine is liberated more slowly from raw soy meal, and equally significant was the fact that heat processing increased the rate of methionine liberation to a greater extent than leucine or lysine.

Studies on the rate of liberation of a specific amino acid from a protein were continued in the case of lysine and the protein casein. Excessive heating of milk protein causes a decrease in its biologic value without greatly affecting its lysine content as determined after complete hydrolysis. It will be recalled that lysine addition corrects the deficiency in biologic value caused by excessive heating of some proteins. Application of the *in vitro* digestibility test showed that the release of amino acids from excessively heated casein was delayed, and that this was especially marked for the amino acid lysine.

As a result of these studies, Melnick and Oser believe that differences in the biologic value of a given protein as a result of heat processing may be referred to the *rate* of release of the constituent amino acids during digestion. More specifically, the essential amino acids, methionine and lysine, are released at different rates relative to other amino acids depending on the extent of heat treatment in the case of certain selected protein foods. Delayed release of an essential amino acid from a protein provides a less efficient amino acid mixture for the tissues for protein synthesis and nitrogen retention.

The biologic value of a protein remains the ultimate test of its efficiency, but in the case of an individual protein food the proposed *in vitro* digestibility test may provide a simpler test for the effect of heat processing on the protein nutritive value of the product. As such it may prove to be of interest and value in food processing.

TUMOR GROWTH INHIBITION BY A PURINE ANALOGUE

Structural analogues, or substrate competitors, have found applications in many fields of research, and the principle involved offers many years of productive research in providing better drugs, bactericidal compounds, insecticides, and fungicides. The usefulness of structural analogues is of course limited by the relative toxicity of the compound to host and parasite, but at least in the case of these compounds some foresight of selection is provided by a study of the metabolism and nutritional requirements of the host and parasite, respectively.

Since the pattern of metabolism is generally similar, even in widely separated species in the phylogenetic scale, it may be rare to find a metabolic antagonist that is completely indifferent to the host. In general, however, it has been noted that the antimetabolite is particularly effective if it cannot be synthesized by the organism, but must be provided in the medium or diet. Hence if the metabolite could not be synthesized by the parasite but could be synthesized by the host, a useful differential toxicity might be provided. Even if both host

and parasite require the given metabolite, some differential might nevertheless be provided by a greater need of the parasite for growth.

Many antimetabolites have of course been synthesized and cannot be reviewed here. A high proportion of these have been structural analogues of vitamins, but others have been prepared, among which are analogues of the purines. To review briefly the biochemistry of the purines, it will be recalled that the purines, adenine and guanine, are essential components of nucleic acids which occur both in the nuclei and protoplasm of all cells. In the mammal, these purines appear to be readily synthesized, with adenine the primary purine from which guanine can be made. In the case of the animal microorganism Tetrahymena geleii, G. W. Kidder and V. C. Dewey (*Arch. Biochem.* **8,** *293 (1945)*) have shown that purines must be supplied in the medium, and furthermore that guanine is a primary requirement (*Proc. Nat. Acad. Sci.* **34,** *566 (1948)*).

In a further study, G. W. Kidder and V. C. Dewey (*J. Biol. Chem.* **179,** *181 (1949)*) have tested a series of twenty-one different substituted purines on the growth of Tetrahymena. Without going into great detail, it was found that some of the analogues tested, for example 1-methyl-guanine, were quite effective in replacing guanine requirements; others, for example, adenine and hypoxanthine, exerted a sparing action; and others exerted an inhibitory action on growth. Of special interest in the latter group was a compound which differs structurally from guanine only in that the carbon atom of the N-C-N sequence in the 5-membered ring portion of the guanine molecule is replaced by nitrogen. Such types have been termed "triazolo" compounds.

The triazolo analogue of guanine was a powerful growth inhibitor of Tetrahymena, possessing an inhibition index of 0.075 (1 molecule inhibits 13 to 14 molecules of guanine). The action of this inhibitor was completely reversed by addition of guanine to the medium. Presumably guanine is utilized in the synthesis of nucleosides and nucleotides and the latter incorporated into nucleic acid. Either the triazolo inhibitor prevents synthesis of nucleotide or is itself actually incorporated to produce an unnatural nucleotide.

The investigators suggest that if pathogenic organisms such as blood and tissue flagellates, intestinal amebas, malarial parasites, viruses, and rickettsias should be similar to Tetrahymena in requiring guanine, then the triazolo analogue of guanine may be a useful drug with possibly little toxicity for the mammalian host. In preliminary experiments the compound was not toxic for mice and it did not affect the fatal course of a single infection tested, namely, that caused by Trypanosoma rhodesiense.

In a later paper, G. W. Kidder, V. C. Dewey, R. E. Parks, and G. L. Woodside (*Science* **109,** *511 (1949)*) extended their interest in the triazolo analogue of guanine to the problem of cancer. Other metabolism inhibitors have been used to slow down or stop the growth of cancer but with limited usefulness, and with difficulties of toxicity to the host. The investigators postulated that if neoplastic tissue has lost its ability to synthesize guanine, then the triazolo analogue ("guanazolo") might be a specific inhibitor for neoplastic tissue. As mentioned earlier, it showed no toxicity for mice when administered over a three day period. A study of the effect of guanazolo on neoplastic growth also seemed justified on the basis of the quantitatively greater need of neoplastic tissue for nucleic acid synthesis.

In their first experiment, a mouse adenocarcinoma was established in each of a group of 18 mice, and the mice then divided into three equal groups. One group received 500 micrograms of guanazolo subcutaneously twice daily, the second group received the same dosage of "diaminazolo" (a less powerful guanine inhibitor), and the third group received injections of saline. The sizes of the tumors were estimated on alternate days and

averaged for each group. The results were striking and showed a marked inhibition of tumor growth by guanazolo. Thus at the end of twelve days the average tumor size of the saline control group was about 11 cm.^{3}, that of the diaminazolo-treated group about 8 cm.^{3}, but only about 1 cm.^{3} in the guanazolo-treated group. Tumor size in fact remained practically stationary during the twenty day treatment period in the latter group. After cessation of treatment the tumors promptly resumed growth. This experiment was confirmed with a larger number of mice, subcutaneous and intraperitoneal administration of guanazolo giving the same results.

A similar striking effect of guanazolo was found on the growth of spontaneous mammary cancer in mice. A mouse lymphoid leukemia was also treated with guanazolo. Using groups of 10 treated and untreated mice, low white blood cell counts, percentages of lymphoblasts, and number of palpable tumor masses were maintained in the treated animals in contrast to the usual picture in lymphoid leukemia. Although one sample of guanazolo produced a leukopenia, this was considered to be due to an impurity since another sample was administered to normal mice for a period of four weeks without producing leukopenia.

The investigators feel that their results show that the malignant cells tested have a changed guanine metabolism, rendering them susceptible to the guanine analogue. This of course invites further work on the purine metabolism of tumor tissue and encourages further research on possible qualitative biochemical differences between normal and neoplastic tissue. The report of Kidder and co-workers has aroused much interest in centers of cancer therapy. It is pointed out that the tumors are only arrested and not killed, and that they resume growth upon withdrawal of the growth inhibitor. Long-continued administration of the drug might of course lead to eventual death of the neoplastic cells or render them more susceptible to other agents, but might also be accompanied by problems of chronic toxicity. In any event the results of Kidder and co-workers are especially noteworthy and surely illustrate the contributions that modern nutrition, biochemistry, and organic chemistry can be expected to make in medical fields.

REDUCTION OF FERRIC TO FERROUS IRON IN THE HUMAN STOMACH

When administered orally in identical fashion, ferrous compounds are more rapidly absorbed from the gastrointestinal tract and produce a more rapid hemoglobin response in iron deficiency anemia than ferric compounds. In addition, ferrous salts are much more soluble than the corresponding ferric compounds. These facts suggest that the ferric ion is reduced in the digestive tract to the ferrous form prior to absorption.

S. L. Tompsett (*Biochem. J.* **34,** *961 (1940)*) has suggested that iron is reduced by the foods in the stomach. He supported this contention with *in vitro* demonstrations of the reduction of ferric iron by certain foods when treated with pepsin-hydrochloric acid solutions. Accurate determination of the extent to which ferric iron was reduced was dependent upon a satisfactory method for the analysis of ferric ion in the presence of the ferrous ion which was developed by E. R. Kirch, O. Bergeim, J. Kleinberg, and S. James (*J. Biol. Chem.* **171,** *687 (1947)*).

In this procedure, the sample was analyzed for ferric iron under conditions in which the ferrous iron would not interfere and for total iron after oxidation of the ferrous iron to ferric by a persulfate solution. The concentration of ferrous iron in the original sample was the difference between the total and ferric ion values. The method was used to determine the degree of reduction and of complex formation by various foods and biologic materials with ferric iron under conditions of

artificial gastric digestion during a three hour incubation period.

Fresh vegetables and fresh fruits reduced as much as 77 to 98 per cent of the ferric iron present. Ascorbic acid was at least partially responsible for this degree of reduction. Egg white, meat, and bread reduced the ferric iron to the extent of about 25 to 40 per cent. Milk gave less reduction and egg yolk none. Sugar produced little effect, but proteins and their digestion products played some role in the reduction of ferric iron by some foods.

O. Bergeim and E. R. Kirch (*J. Biol. Chem.* **177**, *591 (1949)*) have tested the above results in 10 normal human subjects. Tests were made, in the morning, on a fasting subject or five hours after the previous meal. The gastric residuum was removed by aspiration and any traces of food washed out by water. Samples of food of known weight were ingested in routine fashion. In some trials, the individual foods were given without added iron; in other cases, ferric chloride was added in amounts of 0.2, 2, 4, 20, and 100 mg. For some tests, 50 ml. portions of gastric contents were removed every thirty minutes for one or two hours. In other experiments, the entire gastric contents were removed thirty minutes or sixty minutes after ingestion. Samples were filtered and analyzed immediately for ferric and total iron.

Significant reductions from the ferric to the ferrous ion were observed in a variety of foods comparable in character to those reductions obtained in *in vitro* gastric digestion. The reduction degree was the same for ferric iron, which was originally present in the foodstuffs, or that added to the food experimentally. The percentage reduction was independent of the amount of ferric iron present up to 20 mg. per sample. At 100 mg., there was some reduction in the percentage of ferrous iron formed but with an actual increase in the absolute amount of ferrous iron.

In general, reduction values were somewhat higher than those observed with similar foods digested in an artificial medium. As much as 77.8 per cent of the ferric iron ingested in orange juice was reduced, 91.6 per cent of that in tomatoes, 20 per cent of that in peas, and 52.8 per cent of that originally present in boiled potatoes. Foods such as meats gave values as high as 53.5 per cent reduction. The results with milk, whole eggs, and egg yolk were rather irregular. In some experiments no reduction was observed with these materials; in other trials, an appreciable reduction was observed with milk, whole eggs, and egg yolk. Egg white alone always gave fairly high values for reduction of ferric iron. The formation of insoluble complexes of ferric iron with protein, phosphates, and other substances was believed to be one cause for these variations.

An interesting observation was made in connection with the gastric residuum. When this material was incubated alone with ferric iron, amounts of iron corresponding to from 18.0 to 76.5 per cent of the original were reduced during incubation periods of one and two hours. The amount of iron reduced by the residuum appeared to be independent of the pH of the material.

Oxidation-reduction potentials were calculated in a number of the *in vivo* experiments and were somewhat lower than the values obtained for the previous *in vitro* trials. All values obtained were well below +747 millivolts. the oxidation-reduction potential for the ferric-ferrous system, compared with the standard hydrogen electrode.

The data described above seem to demonstrate definitely that, in man, the stomach is an important site for reduction of ferric to ferrous iron. Further studies are necessary in diseases where the normal digestive and secretory abilities of the stomach are deranged or lacking.

THIAMINE REQUIREMENT OF INFANTS

Relatively little quantitative information exists on the thiamine requirement of infants, although the thiamine requirement of adults has been the subject of much study. The Food and Nutrition Board of the National Research Council ("Recommended Dietary Allowances," *National Research Council, Reprint and Circular Series No.* **122** (*1945*)) has recommended 0.5 mg. of thiamine per 1000 calories for infants, children, and adults alike. Their recommendation for infants and children was based largely on the work of F. W. Schlutz and E. M. Knott (*J. Nutrition* **15,** *411* (*1938*); *Am. J. Dis. Child.* **61,** *231* (*1941*)). These reports include a fairly extensive study of children on various diets and under various conditions. Their data on infants, however, are scanty. Since infants are known to have unusual needs for many nutrients, it is possible that their thiamine requirement might be different from children or adults.

L. E. Holt and co-workers (*J. Nutrition* **37,** *53* (*1949*)) have added considerable information on this subject. Their studies were conducted on 7 healthy male infants from 1 to 10 months of age. A purified diet was employed which consisted of vitamin-free casein, vitamin-free Dextri-maltose, hydrogenated cottonseed oil, and a special salt mixture. These constituents were mixed with water to form a paste, cereal-like in consistency. The diet was so compounded that 50 per cent of the calories were supplied by carbohydrate, 35 per cent by fat, and 15 per cent by protein. One hundred calories per kilogram of body weight were fed daily. All of the vitamins were given as supplements. Vitamins A and D were given as oleum percomorphum, 15 drops daily. The water soluble vitamins were given midway between meals in four equal doses each day to yield a daily intake of riboflavin 0.167, nicotinamide 4.16, pyridoxine 0.167, calcium pantothenate 0.167, para-aminobenzoic acid 0.167, choline 0.833, inositol 0.167, and ascorbic acid 4.0 to 12.0 mg. Thiamine was given in variable amounts.

The thiamine requirement was determined using the "point of minimum excretion" technic. This method is based on the fact that thiamine excretion in the urine decreases as a linear function of the intake until a certain critical point is reached, i.e., the "point of minimum excretion." Decreasing the intake beyond this point causes only a minimum further decrease in thiamine excretion. According to their view, the thiamine excreted above this critical point represents a surplus above minimum physiologic needs. Below this point, clinical or subclinical deficiency is apt to occur. The authors have found confirmation of these ideas in their own work and in the work of others with both man and animals.

In 5 subjects, after two periods on a high thiamine intake (1.667 and 0.667 mg. per day), the thiamine was withdrawn entirely for fourteen to twenty-one days. Two additional subjects were started immediately on zero thiamine intake without the preliminary periods of high thiamine. When the urinary thiamine excretion stabilized at a low level, oral thiamine was added cautiously in small amounts increasing the intake at intervals of a week or two until excretion beyond the minimum occurred. The thiamine intake was then adjusted until an amount was found which would maintain urinary excretion just above the minimum point.

The results were remarkably uniform. The minimal thiamine excretion in their infants corresponded to a urinary output of 1 to 5 micrograms per twenty-four hours. The daily thiamine intake which produced an excretion just in excess of this minimal level was 0.14 mg. in one subject, 0.16 mg. in 2 others, and 0.20 mg. in the remaining 4. Reduction of intake below these amounts invariably resulted in an output of less than 5 micrograms per day in the urine, whereas the

above quantities would maintain an output of between 5 and 10 micrograms per day. Any further increase produced a sharp rise in thiamine excretion with about one quarter of the added thiamine appearing in the urine. From these results they concluded that the thiamine requirement of their infants varied between 0.14 and 0.20 mg. per day.

Though their subjects were maintained for four to five months on a thiamine intake close to what they regarded as the minimum, no signs or symptoms of thiamine deficiency developed as ascertained by careful clinical and laboratory examinations. However, after about six months on the purified diet, a diminution or cessation of weight gain occurred. This was not related in any way to thiamine since thiamine therapy would not affect it nor was it prevented when adequate thiamine was given from the beginning of the experiment. The addition of yeast could correct it in the majority of the cases. This phase of their work has been continued and expanded as reported elsewhere (S. E. Snyderman, R. L. Nemir, and L. E. Holt, *Federation Proceedings* **8**, *397* (*1949*)). Whether this additional deficiency complicated their results on thiamine requirements is not clear.

It is of interest to compare the minimum thiamine requirement, as determined by their technic, with the intake the average artificially-fed infant receives. Based on an average content of 0.035 to 0.04 mg. of thiamine per 100 ml. of cow's milk, an infant weighing 7 kg. would receive at least 0.30 mg. a day, apparently an ample margin of safety beyond a daily requirement of 0.20 mg. On the other hand, destruction of thiamine by heat in the pasteurization and sterilization of milk formulas must be considered.

According to S. K. Kon (*Brit. Med. Bull.* **5**, *170* (*1947*)), 10 per cent of the thiamine may be destroyed by the former process and 30 per cent or more by the more drastic sterilization procedures. The margin of safety with sterilized milk would thus appear to be small indeed, and the possibility that autoclaving milk, which is becoming increasingly popular, may destroy even more thiamine is one that demands careful study. Since breast milk contains roughly half as much thiamine as cow's milk, the margin of safety it provides would seem to be decidedly small.

Comparison of the thiamine requirement, as determined in this study, with the recommendations of the National Research Council, is also of interest. Using the latter recommendation, the infants studied by Holt *et al.* (*loc. cit.*) would have been fed from 0.20 to 0.38 mg. of thiamine per day. Although this is somewhat more than these authors found to be necessary, their data do support the recommendations of the National Research Council if a reasonable allowance for a margin of safety is made.

Many factors are known to influence thiamine requirements and a considerable margin of safety would seem desirable. It should be remembered, too, that the data of Holt *et al.*, although collected carefully and well controlled, were obtained under artificial dietary conditions. The authors emphasized that their data on thiamine requirements applied only under the conditions of their experiments. It is nevertheless of considerable interest that their figures do agree so closely with data derived from previous studies using different technics.

FURTHER STUDIES ON DIET AND TUMOR GROWTH

Extensive studies over a period of many years have indicated that changes in diet may profoundly influence the growth of tumors (*Nutrition Reviews* **4**, *353* (*1946*)). The problems presented are complex, however, since there are not only many kinds of tu-

mors, some arising spontaneously and some requiring transplantation, but also these tumors may arise or be induced in the many tissues of animals of many species and, finally, the number of nutrients in the diet which may be varied is, of course, large. Diverse results have been reported from various laboratories. For example, whereas riboflavin stimulates the growth of the Rous sarcoma in chicks the same vitamin inhibits the development of liver tumors in rats fed azo dyes (*Ibid.* **7,** *82* (*1949*)).

Experiments are reported by A. Tannenbaum and H. Silverstone (*Cancer Research* **9,** *162* (*1949*)) in which only one species of animal was used, the mouse. The proportion of protein in the diet was varied by an inverse change in the quantity of carbohydrate, thus actually two components of the diet were varied. The tumors studied were spontaneous mammary carcinomas, skin tumors, and sarcomas induced by carcinogenic hydrocarbons and hepatomas appearing spontaneously in one strain of mice.

The only change in incidence of tumors which was observed to be related to the changes in diet was that of the spontaneous hepatomas. With these tumors the incidence was lower when a 9 per cent casein diet was fed than when one containing 18 per cent casein was given. In no other instance did the changes in the diet affect the incidence or time of appearance of the tumors. The mice on the diets of various protein content grew at similar rates except that the diet containing 18 per cent protein produced a little better weight gain than that containing other quantities varying from 9 to 45 per cent.

A. C. Griffin, C. C. Clayton, and C. A. Baumann (*Cancer Research* **9,** *82* (*1949*)) found results somewhat different from those of Tannenbaum and Silverstone. In their experiments, rats were fed several azo dyes, and the incidence and time of development of liver tumors observed. In contrast to the results of Tannenbaum and Silverstone, with spontaneous hepatomas in mice, the incidence of tumors in rats fed azo dyes was usually less when 24 per cent rather than 12 per cent casein was included in the diet. In these studies the animals fed the dyes did not grow nearly as well on the low protein diet as they did when the concentration of protein was higher.

General well-being and survival were also improved by the higher levels of dietary protein. The effects of the protein were more marked in experiments in which a high concentration of a moderately carcinogenic dye was fed than when a low concentration of a very potent dye was fed. The protective effects of protein against tumor formation were therefore regarded as being primarily due to an increased resistance of the rats to a toxic compound rather than to any specific anticarcinogenic property of protein or methionine.

In another experiment by these investigators, two other dietary variables were changed, riboflavin and methionine. In general, riboflavin appeared to prevent the development of tumors when low levels of casein were fed. This is in agreement with studies previously reported from this laboratory (*Nutrition Reviews* **4,** *353* (*1946*)). When methionine was added to the low casein diet, that is to the 12 per cent casein diet, in quantities sufficient to bring the methionine concentration up to that contained in the 24 per cent casein diet, there was in some instances a decrease in the incidence of hepatomas, but this was not as clearcut as were those following the increase in dietary protein alone.

Analysis of the livers of the rats fed azo dyes indicated that both casein and methionine improved the retention of riboflavin in this organ. It is possible that this effect partially explains the inhibiting effect of the casein and, to a lesser extent, of methionine. Previous work from the same laboratory indicated that the azo dye became closely bound to protein in the liver and that this binding was important in the pathogenesis of the tumors (*Nutrition Reviews* **6,** *217*

(*1948*)). Furthermore, inclusion of large amounts of riboflavin in the diet not only retarded carcinogenesis but also decreased the amount of bound dye found in the liver. Thus the effectiveness of casein and of methionine in increasing the riboflavin content of the liver might in this way reduce the amount of bound dye and retard carcinogenesis.

In another study concerned with chemically induced cancer, C. G. Tedeschi (*Arch. Path.* **47**, *160* (*1949*)) observed the effect of changes in the diet upon the incidence and rapidity of growth of embryonal liposarcomata. Mice were injected into the subcutaneous fat with 0.3 ml. of 5 per cent 1,2,5,6-dibenzanthracene in corn oil. Three diets were used: the "standard" composed of mice biscuits ad libitum, a low caloric diet containing only one-third the quantity of biscuits given on the standard, and a high caloric diet made by adding one slice of bacon daily.

The mice on the low caloric diet lost weight but gained on the high caloric diet. However, there was no influence on tumor incidence, growth, or structure. The number of animals was small so that minor changes might not have been picked up. There were 14 mice each in the groups receiving the high and low caloric diet and 10 on the control diet.

Another group of 10 mice maintained on the standard diet was given the injections of the carcinogen in pork fat rather than corn oil. Twice as many animals developed tumors with the corn oil as solvent as when pork fat was used. Furthermore the tumors appeared earlier when corn oil was used. The authors suggest that the rate of absorption of the carcinogen has some influence on its effect, although another possibility is a stabilizing effect of the antioxidants in the corn oil on the carcinogen. Hydrocarbons disappear from autoxidizing lipid mixtures, and this disappearance can be delayed markedly with tocopherol.

MYOCARDIAL LESIONS IN EXPERIMENTAL VITAMIN E DEFICIENCY

In early studies on vitamin E deficiency, the gross signs and the histologic appearance of dystrophy of the voluntary muscles were described with considerable clarity without report of any cardiac involvement (M. Goettsch and A. M. Pappenheimer, *J. Exp. Med.* **54**, *145* (*1931*); C. G. Mackenzie and E. V. McCollum, *J. Nutrition* **19**, *345* (*1940*)).

Evidence that vitamin E deficiency in rabbits was associated with myocardial damage was presented by O. B. Houchin and P. W. Smith (*Am. J. Physiol.* **141**, *242* (*1944*)) who noted that dystrophic rabbits had an increased sensitivity to pitressin, a greater resistance to the toxic effects of cardiac glucosides, and probable cardiac dilatation as revealed by thoracic radiographs. In addition, slices of cardiac muscle from dystrophic rabbits had an increased oxygen consumption. They concluded that the sudden death of vitamin E deficient rabbits in an advanced stage of muscular dystrophy was due directly to myocardial failure.

In chronically vitamin E deficient rats, K. E. Mason and A. E. Emmel (*Anat. Record* **92**, *33* (*1945*)) described the appearance of characteristic pigment globules in cardiac muscle fibers along about the tenth or twelfth month of the deficiency. The pigment globules appeared as linear groups located irregularly in the sarcoplasm. Later, more definite histologic injury became apparent. Actual necrosis of cardiac fibers was less evident but fibrotic replacement of the fibers was more marked than in skeletal muscle. The peripheral portion of the ventricles showed the most marked lesions, but the interventricular septum and the musculature of the auricles were also affected.

Since a considerable percentage of the experimentally vitamin E deficient cattle died suddenly and without demonstration of evident cause in gross postmortem examination, T. W. Gullickson and C. E. Calverley (*Science* **104,** *312* (*1946*)) began to make electrocardiograms at intervals of one month, or oftener, on all cattle on vitamin E deficiency trials. Study of the series of electrocardiograms obtained from the first heifer to die after beginning this type of observation indicated a gradual and progressive change as the deficiency progressed. The first abnormality consisted of an increase in the PR interval which persisted for the four months prior to death, followed by changes in the QRS interval and changes in the potential of various deflections. In general, these changes in the electrocardiograms could be considered as indications of a decreased functional activity of the myocardium in the terminal stages of vitamin E deficiency. Definite abnormalities were seen in histologic preparations, with atrophy and scarring of the cardiac muscle fibers and an increase in the cellular elements.

Later, A. J. Gatz and O. B. Houchin (*Anat. Record* **94,** *462* (*1946*); **99,** *578* (*1947*)) briefly described certain cardiac changes which occurred in vitamin E deficient rabbits. In somewhat greater detail, J. H. Bragdon and H. D. Levine (*Am. J. Path.* **25,** *265* (*1949*)) studied the relation of vitamin E deficiency to myocardial abnormalities in rabbits. Young male and female white rabbits were offered either of two simplified vitamin E deficient diets composed primarily of vitamin-free casein, lard, sucrose, and salt mixture, supplemented with thiamine, pyridoxine, riboflavin, calcium pantothenate, niacin, choline chloride, vitamin A, and vitamin D. The diets differed from each other in fat content, the one containing 20 per cent lard, and the other only 10 per cent. Each rabbit also received 0.5 ml. of refined corn oil three times a week. The control rabbits received 15 mg. of alpha-tocopherol in each 0.5 ml. of corn oil.

Of the 19 rabbits receiving the vitamin E deficient diets, 17 survived long enough to develop muscular dystrophy. The 5 animals fed the higher fat diet failed to gain weight, developed dystrophy, and died on the average in the sixth week. The 12 animals which received the lower fat diet gained weight for about four weeks, weakened, and were either killed or died, on the average, in the seventh week. None of the 6 control rabbits, 3 on each diet, developed muscular dystrophy. Four gained weight until killed after six or eight weeks on experiment. The other 2 showed a significant loss of weight during the terminal weeks, presumably due to coccidial cirrhosis demonstrated at necropsy.

Electrocardiograms were taken only on rabbits receiving the lower fat diet. Seven of the deficient animals had two or more tracings, one before the animal was offered the experimental diet, and another prior to being killed. The later tracings of 5 of these 7 rabbits showed changes that were interpreted as definite abnormalities, including elevation of the S-T segment and inversion of T waves in lead II. These changes developed late in the period of observation after muscular dystrophy had become severe. No similar changes were observed in the controls nor in 23 normal rabbits maintained on other diets.

All 17 rabbits which developed dystrophy on the deficient diets showed the series of typical gross and microscopic changes in the skeletal muscles described originally by M. Goettsch and A. M. Pappenheimer (*J. Exp. Med.* **54,** *145* (*1931*)). In addition, 15 of them had definite foci of necrosis, accompanied by inflammatory reaction in the myocardium. The sequence of morphologic events in cardiac muscle resembled that in voluntary muscle and appeared to be as follows: "a coagulative necrosis of sarcoplasm with loss of striations; pyknosis and later caryorrhexis of muscle nuclei; and an inflammatory reaction characterized by edema, small hemorrhages, and the infiltration of monocytes and polymorphonuclear

neutrophils in varying proportions." Calcification in cardiac lesions was observed in some areas, usually where there was calcification of necrotic voluntary muscle as well. Myocarditis was observed to be consistently more extensive in the rabbits receiving the higher fat diet where the vitamin E deficiency was more severe.

No effort to study the effect of vitamin E therapy upon cardiac lesions was described in these investigations. In view of the current interest in the evaluation of possible beneficial effects of vitamin E and its derivatives in human coronary disease, studies on the ability of cardiac lesions in experimental vitamin E deficiency to heal when vitamin E is administered are the logical sequelae to the investigations described above.

There appears to be insufficient evidence yet to justify formulation of any hypothesis that human coronary disease and the experimental lesions described herein have a common origin. The stringent and prolonged deficiency of vitamin E necessary to produce cardiac lesions in experimental animals seems unlikely to occur in man unless there are unrecognized high metabolic demands for vitamin E or inabilities to use the forms of vitamin E normally provided by the human dietary. That similarities do exist between the types of cardiac lesions in experimental vitamin E deficiency and those in human coronary disease suggests the need for thorough investigation of the various possibilities which might be involved in production of vitamin E deficiency in man.

NUTRITION OF THE GUINEA PIG

Use of the guinea pig as an experimental animal in nutritional studies was initiated early in this century, when it was found to develop a hemorrhagic condition when kept on certain rations deficient in the vitamin now known as ascorbic acid. Despite several investigations in recent years, it is still not possible to devise a purified ration for these animals that will produce a growth rate comparable to that permitted by natural rations.

In an investigation made several years ago, D. W. Woolley and H. Sprince (*J. Biol. Chem.* **157,** *447 (1945)*) described three factors necessary for the guinea pig. One of these was apparently identified as folic acid, another was replaceable by a mixture of cellulose and protein, while the third was an unidentified substance supplied by liver extracts. The best growth obtained when all three of these factors were added to a basal ration was only 4 g. per day for four weeks. This should be compared with the 7 to 8 g. per day from the second to the eighth weeks of life which can be regularly obtained when these animals are fed a good ration of natural foodstuffs (M. D. Cannon *et al.*, *Arch. Biochem.* **7,** *55 (1945)*).

K. A. Kuiken, R. H. McCoy, M. O. Schultze, and C. G. King (*J. Nutrition* **27,** *385 (1944)*) developed simplified rations capable of supporting growth up to 4.4 g. per week through a period of one hundred days, and concluded that guinea pigs required at least two nutrients of a vitamin nature, beyond those identified at that time, one of which was supplied by crude casein (presumably vitamin B_{12}) and another by rice polish concentrate (presumably folic acid).

Additional studies on the nutritional requirements of these animals have now been reported by A. N. Booth, C. A. Elvehjem, and E. B. Hart (*J. Nutrition* **37,** *263 (1949)*). A commercial ration for guinea pigs was used as the control diet, and growth on it was compared with that on a synthetic ration (adequate in protein and all other known nutrients with the exception of vitamin B_{12}) variously supplemented with crude materials.

On the commercial ration, the pigs gained an average of 6.9 g. per day over a six week

period as compared with only 1.8 g. on the synthetic ration. Any of a variety of supplements high in bulk-producing capacity improved growth on this ration when added in amounts comprising up to 30 per cent of the ration. These included powdered cellulose (celluflour), beet pulp, alfalfa leaf meal, pectin, and gum arabic. Gum arabic was one of the best of these supplements. When hydrolyzed with dilute acid, the acid removed, and the hydrolysate fed, it was ineffective.

The apparent importance of bulk in promoting growth of guinea pigs, first recognized by Woolley and Sprince (*loc. cit.*) is thus confirmed, although nothing is yet known with certainty of its function. One of several possible functions would be to create within the intestinal tract and cecum, conditions favorable for the growth of microorganisms which could synthesize food factors essential for the guinea pig, and made available to it either through direct absorption from the tract, or through coprophagy.

When the synthetic diet plus 15 per cent of gum arabic was fed, growth (average about 5.1 g. per day) could be further improved by addition of rather high levels of brewers' yeast or alfalfa leaf meal, but not by many other materials such as grass juice powder, fish solubles, or raw milk, which seemed rather to depress growth. When a variety of organic fractions of alfalfa leaf meal failed to duplicate the growth-promoting action of the intact meal on these rations, an ash was prepared from it and fed. This proved active in stimulating growth. Preliminary investigations revealed that cobalt, boron, molybdenum, sodium, calcium, magnesium, or phosphorus were not effective individually in replacing the ash, and the authors expressed the belief that the effect of the ash might lie in affording a proper balance between certain important mineral elements.

Subsequently, the problem has been further investigated by P. Roine, A. N. Booth, C. A. Elvehjem, and E. B. Hart (*Proc. Soc. Exp. Biol. Med.* **71,** *90 (1949)*), who found that individual additions of substantial levels of magnesium oxide or of potassium acetate to the basal ration containing gum arabic improved growth somewhat. In combination, these two supplements fully duplicated the growth-promoting action of the ash of alfalfa leaf meal.

Addition of 2.5 per cent of potassium acetate and 0.5 per cent of magnesium oxide to the ration permitted growth equal to that obtained on the natural stock ration. The two salts do not produce increased growth on the ration if the gum arabic is omitted, hence it is not known whether they are required directly by the animal in these increased amounts (the basal ration contained both potassium and magnesium in amounts fully adequate for other animals), whether their effect is mediated indirectly through the intestinal flora, or whether they act in still another fashion.

The means by which excellent growth can be attained on purified rations represents a significant advance in knowledge of the nutrition of this species. It should now be possible to assess directly the significance of each component of the ration, and to investigate in detail the significance and mode of action of the "bulk" factors, such as gum arabic.

It is already apparent from the results of Booth *et al.* (*loc. cit.*) that this factor plays a highly significant and unexplained role in sparing the protein requirement of the pigs on these rations. The necessity for a high level of casein (30 per cent) in synthetic diets to obtain growth was recognized by Woolley and Sprince (*loc. cit.*) and confirmed by the present investigators. The latter, however, point out that the commercial stock ration, which is fully adequate, contains only 18 per cent of protein. Although they could not secure good growth when the casein of their ration was lowered to 20 per cent, good growth was obtained with this level in a ration containing 30 per cent of beet pulp

(which supplies only 2 per cent of additional protein) and in which sucrose was replaced by dextrin. The explanation of these results, too, is unknown at present.

These results demonstrate how far from complete is our present knowledge of factors which affect the nutrition of experimental animals. At the same time, they make possible further investigations which should do much to elucidate some of the influences of importance which have not hitherto been well understood.

NUTRIENT REQUIREMENTS FOR ADULT GROWTH IN MAN

With attainment of adulthood, it is frequently assumed that growth ceases in man, and that unless fatty tissues are being deposited, balances of nitrogen, phosphorus, etc., should be essentially zero. However, it is also well known that growth in the adult does not cease entirely. The hair and nails continue to grow throughout life, as does epithelial tissue in general. Of particular significance is the skin, the outer layer of which is constantly being desquamated and replaced from the lower layers.

Other morphologic aspects of adult growth have been described by the anthropologist, A. Hrdlička (*Proc. Am. Phil. Soc.* **76,** *847 (1936)*), as follows: "The majority of the dimensions or characters, headed by stature, progress perceptibly until well into the fourth decade of life; others, including the skull and face, increase slightly, in some groups at least, until the fifth or even the sixth decade; and still others, such as the dorsal length and breadth of the nose, the length of the ears, width of the mouth, depth of the chest, keep on augmenting generally throughout most, if not all, of the adult existence."

These factors make it obvious that slight positive balances of nitrogen, phosphorus, calcium, and other nutrients should be expected throughout life. In an effort to measure the magnitude of these in man, H. H. Mitchell (*Arch. Biochem.* **21,** *335 (1949)*) has reexamined data on the nitrogen and phosphorus balances of 23 young adult men, obtained during investigations conducted over a two hundred and twenty day period during the year 1908. No changes in average weight of the group occurred during this period. However, the nitrogen and phosphorus balances were positive over the entire period, and indicated average daily accumulations of nitrogen and phosphorus of 1.38 and 0.175 g. per day, respectively.

All of this retention cannot be ascribed to adult growth, since no account was taken of losses through the skin during perspiration. On the basis of recent measurements, and when sweating is minimal, these daily dermal losses average roughly 360 mg. of nitrogen and less than 0.5 mg. of phosphorus, leaving positive balances of about 1 g. of nitrogen and 174 mg. of phosphorus per day as the best estimates of the requirements for growth in adults maintaining essentially constant weight. These balances showed a highly significant positive correlation with body surface of individual subjects and with each other.

The magnitude of these positive balances is surprisingly large, that for nitrogen corresponding to over 6 g. of protein per day. The authors believe, however, that they are real, and not due to constant errors in analyses or in the collection of excreta. If the figures are reliable, and are maintained over many years of adult life, it would appear necessary to postulate losses of epithelial tissue, hair, etc., at rates appreciably higher than those which would be expected on *a priori* grounds. Even so, the magnitude of the balances is small in terms of recommended intakes of protein and of phosphorus for adult individuals, and hence are probably not of great importance in reckoning the nutritional requirements of the adult.

COPROPHAGY AND VITAMIN REQUIREMENTS IN THE RAT

It has long been known that the microorganisms of the intestinal tract of animals synthesize many vitamins and food factors. The latter may become available to the host animal in either of two ways: (a) direct absorption from the liquid contents of the intestine through the intestinal wall—a process which undoubtedly operates to supply certain vitamins to the host animal, but the quantitative importance of whi h is generally unknown, difficult to determine, and appears to vary with the diet; and (b) through the phenomenon known as coprophagy, or direct consumption of feces by the animal. The latter process has been frequently observed to occur in rats, for example, and is an effective means of supplying the animal with food factors lacking in its diet (see *Nutrition Reviews* **4,** *116* (*1946*)).

The general use of cages with raised and screened bottoms minimizes, but by no means eliminates this practice, since the rat has been observed to obtain feces pellets as they are excreted (R. P. Geyer *et al.*, *J. Nutrition* **33,** *129* (*1947*)). The latter workers devised a tubular cage which restricted the rat in such a manner that active movements of most kinds, including access to its own excreta, were prevented. Through use of such a cage, it becomes possible to compare the nutritional requirements of rats allowed free movement in the ordinary cage with those of rats allowed limited movement, and in which coprophagy cannot occur. Several such experiments have now been conducted by V. H. Barki and coworkers (*Ibid.* **37,** *443* (*1949*)).

These workers employed a diet adequate in protein, fat, minerals, fat soluble vitamins, calories, and five vitamins: thiamine, riboflavin, pantothenic acid, vitamin B_6, and choline. Such a "synthetic" diet allows good growth in rats under normal conditions. The effects of additions of a mixture of niacin, folic acid, biotin, para-aminobenzoic acid, and inositol were then compared when given to rats caged in the tubular cage, and in the ordinary type of square cage with raised screen bottoms.

It was first observed that confinement in the tubular cages reduced the growth of all the rats when compared with that of their mates on the same diets in the square cages. However, within the square cages, the five extra vitamins improved growth of the rats by only about 10 per cent (from 185 ± 8.8 g. (average gain in weight over six weeks ± standard error) to 207 ± 6.9 g.), while in the tubular cage the same supplement increased the weight gain by 29 per cent (125 ± 3.9 g. to 161 ± 4.7 g.).

Separate experiments were undertaken to determine which of the five supplemental vitamins were required to produce the extra weight gains in the animals confined in the tubular cages. Omission of either biotin or folic acid from this mixture of five vitamins permitted only small gains over the control group; and supplementation with these two vitamins alone permitted weight gains equal to those obtained with the complete mixture. Omission of niacin, inositol, and para-aminobenzoic acid thus had almost no deleterious effects on growth. In contrast, biotin and folic acid produced only slight and irregular growth increases over the basal group when supplied to rats kept in the square cages. These results seem to show that direct deficiencies of biotin and folic acid can be demonstrated in the rat when coprophagy is strictly prevented, a demonstration which cannot be made under ordinary conditions by dietary restriction alone.

Previous investigations (for example, E. Nielsen and C. A. Elvehjem, *J. Biol. Chem.* **145,** *713* (*1942*)) showed that sulfasuxidine increased the dietary requirement of the rat for biotin and folic acid. A comparison of the growth and liver storage of biotin and folic acid was therefore made in rats kept in the tubular cages, and in rats kept in the square cages but fed sulfasuxidine. Under the con-

ditions of the experiment, growth of the two groups was approximately equal, but less than that of the control group. In both groups, the folic acid and biotin contents of the liver were much lower than those found in a control group housed in square cages and fed the biotin and folic acid-free ration without sulfasuxidine.

All of these results tend to substantiate previous conclusions that both folic acid and biotin are dietary requirements of the rat, although they need not ordinarily be present in the diet because they are supplied by intestinal synthesis. Coprophagy would appear to be an important mode of obtaining these vitamins in the rat. However, it must be pointed out that even with the best diets, growth in the tubular cages has not equaled that in the square cages. This fact suffices to show that the rats thus caged are not fully comparable to those housed in the customary fashion, and the demonstration that they respond to dietary supplements of biotin and folic acid under these abnormal conditions is, therefore, not fully convincing evidence that they would do so under "normal" conditions, even if coprophagy could in some way be prevented.

FOOD PROCESSING AND NUTRITIONAL VALUES

A large portion of the nation's food supply is subjected to one or another type of industrial processing before it reaches the consumer. It is obvious, therefore, that the effect of this processing on the nutritive value of the foodstuff is of concern to public health workers. Accordingly, there have appeared two partial summaries of these effects on grain foods (F. W. Nordsiek, *Am. J. Pub. Health* **39,** *751 (1949)*) and on canned foods (E. J. Cameron, R. W. Pilcher, and L. E. Clifcorn, *Ibid.* **39,** *756 (1949)*).

The paper by Nordsiek on the processing of grain foods is of value because of the insight which it provides into the basic procedures used in preparation of grains for food and the alterations induced by these steps. Nordsiek divides these preparative steps into four basic procedures: (1) milling; (2) manufacture; (3) restoration of nutrients to original levels; and (4) supplementation with nutrients to augmented levels.

This writer points out that all but 2 per cent of the wheat flour manufactured in the United States is milled white flour. The process of milling removes the outer chaffy husk from the grain kernel, separates the bran coats and the germ, and leaves the endosperm. The latter may then be left whole (rice), broken into coarse particles (corn grits), or ground (meals and flours). It is apparent that straight milling results in the loss of those nutrients present in the outer coats and germ of the grain. By undermilling, a portion of the bran and germ may be retained in the food product, giving rise to so-called "long-extraction" flours. Methods such as the preparation of parboiled rice exist for the transfer of nutrients from the outer layers to the endosperm prior to milling (*Nutrition Reviews* **7,** *125 (1949)*).

Manufacturing generally includes conversion of the milled cerealstuff into the product to be marketed, such as preparation of bread, baked goods, macaroni, and breakfast cereals. Thus, the process of manufacture may increase the nutritive value of the cereal product because of the addition of such nutritious ingredients as milk, milk products, eggs, shortening, yeast, and the like. On the other hand, manufacture may result in a reduction in thiamine content, due to the process of heating (baking of bread, toasting, and the like). The heating process may either increase or decrease the biologic value of the protein of cereals used in the preparation of breakfast foods (*Nutrition Reviews* **3,** *238 (1945)*).

Restoration of some of the nutrients (the third basic operation in cereal processing) may be carried out by addition to flour at the mill of a vitamin-mineral premix or the use of "enrichment wafers" by the baker in order to produce enriched bread from unenriched flour. Grain foods of large particle size (corn grits, rice) may be enriched by mixing with particles of the food which have been "super-enriched." These "super-enriched" particles are distributed through the food product and serve as carriers of the added vitamins and minerals.

Supplementation to augmented levels of nutrients may be brought about by addition of synthetic vitamins, vitamin concentrates, or minerals to levels higher than those which are found in the original grain. In practice, such levels are reached in the supplementation with riboflavin and the optional ingredients, calcium and vitamin D.

The net result of these four processing steps consists of removal of nutrients present in the germ and outer layers of the cereal grain, loss or other alteration of nutrients which accompany manufacture, and restoration to whole grain levels or above of thiamine, riboflavin, niacin, iron, and, in some instances, calcium. It remains impossible to define with certainty all gains or losses which may occur during processing. Some of the implications of processing have been previously discussed (*Nutrition Reviews* **7,** *88 (1949)*).

Another industrial process of importance is canning. Cameron, Pilcher, and Clifcorn (*loc. cit.*) summarize illustrative data on the over-all vitamin retention during the canning procedure and specific retentions during particular operations, especially blanching. This whole problem has been considered in detail in two reviews in this journal (*Nutrition Reviews* **7,** *142, 144 (1949)*) and, accordingly, will not be presented again here. Suffice it to point out that, in general, over-all vitamin retentions during canning were rather high, except for occasional foods. There occurred considerable variability in retention—most marked in those foods where retention was less satisfactory (for example, green beans and lima beans).

The operation of blanching accounts for a large portion of nutrient loss. Studies indicate that conversion to high temperature, short-time blanching procedures may be expected to improve retention of heat-labile vitamins. As previously noted (*Nutrition Reviews* **7,** *144 (1949)*), low-temperature storage of canned foods is important in preservation of nutritional values over long periods of time.

It is reassuring to note the keen interest of the food industries in development of processes designed to result in processed foods of dependable nutritional quality. Retention of nutritive values alone is but one of the problems of concern to the food processor desirous of providing a wholesome food. It has long been recognized that food must be free of harmful microorganisms, and dependable precautions in this direction have been at the root of the processed food industry. Recently the food industries have become alerted to another general type of problem; namely, the necessity for protection of food products against contamination with toxic residues of pesticides or other chemical technologic aids.

Several thousand brands of new insecticides, fungicides, and herbicides (weed killers) have appeared on the market in the past few years. In many instances, relatively little is known concerning the acute or chronic pathologic effects of these substances on man. The residues of some of these new poisons are difficult to remove from food products. Others are incorporated in the plant tissues and cannot be removed. It is apparent, therefore, that the food processor as well as the farmer today is faced with the problem of preventing potentially toxic quantities of these substances from being carried through into processed foods. Oftentimes he is at a disadvantage because of the

inadequacy of methods for testing for the given pesticide. As pointed out in an editorial (*J. Am. Med. Assn.* **137,** *1604 (1948)*), "The recognition of the toxicologic problems presented by the contamination of foods and beverages through the use of materials for the purpose of combating pests and the use of chemical technologic aids for improving the appearance, texture, flavor, or lasting qualities of foods may well be the beginning of a new era in the science of foods and nutrition."

FAILURE OF TETRAHYMENA GELEII TO UTILIZE TRYPTOPHAN IN PLACE OF NIACIN

An extended series of investigations by Kidder and Dewey concerning the nutritional requirements of the unicellular protozoan, Tetrahymena geleii, has appeared within recent years. These are of particular interest since this organism, like other protozoa, belongs formally to the animal world, and a comparison of its nutritional requirements with those of higher animals becomes of importance to comparative biochemistry. Previous investigations have established that the organism requires many of the vitamins and amino acids required by higher animal forms.

The summarized results of many different investigations show conclusively that tryptophan, in adequate amounts, eliminates the requirement of higher animals (the rat, pig, dog, chick, and man are among the species so far investigated) for niacin by itself serving as a precursor from which niacin is synthesized (see *Nutrition Reviews* **6,** *50, 77, 252 (1948)*). All of the evidence points, furthermore, to the view that the animal tissues themselves carry out this transformation, and that it is not mediated exclusively by intestinal microorganisms. It was of interest, therefore, to determine how the simple animal organism, Tetrahymena geleii, would behave.

For this purpose, G. W. Kidder, V. C. Dewey, M. B. Andrews, and R. B. Kidder (*J. Nutrition* **37,** *521 (1949)*) grew Tetrahymena geleii in a defined medium, supplemented with varying levels of a mixture of amino acids, tryptophan, and niacin. In accordance with earlier results, the organism was found to require niacin for growth even though tryptophan was present; furthermore, an increase in the tryptophan concentration did not spare the niacin requirement, but if anything, tended to enhance it slightly. Unlike higher animals, therefore, this unicellular animal has lost the ability to synthesize niacin from tryptophan, and requires the vitamin preformed. In this respect, it resembles several lactic acid bacteria, yeasts, and Proteus vulgaris, which are classified formally as plants and which also require preformed niacin for growth (B. E. Volcani and E. E. Snell, *Proc. Soc. Exp. Biol. Med.* **67,** *511 (1948)*), more than it resembles the higher animals.

However, there are other animal forms which are incapable of converting tryptophan to niacin, for example, germ-free cultures of Drosophila (J. Schultz and G. T. Rudkin, *Federation Proceedings* **7,** *185 (1948)*). It thus becomes apparent that the mere fact that two organisms are both animal forms, although widely separated on a phylogenetic basis, is no guarantee that their nutritional requirements will be similar in detail. Indeed, variations from simple to complex nutritional requirements have been observed among various groups of protozoa as it has been among various groups of bacteria, and from the mutational origin of nutritional requirements such diversity would be expected.

NOTES

Nutritive Value of Cultured Milk

In many countries, particularly in Scandinavia, natural buttermilk or artificially fermented milk is used as an important food. The flavor is enjoyed better than that of sweet milk by many populations and the keeping qualities are, of course, considerably better. Two studies have been reported from Finland concerning the nutritive value of fermented milk.

P. Roine and T. Z. Csaky (*Suomen Kemistilehti* **21,** *(B) 48 (1948)*) studied the nutritive value of cultured buttermilk on rat growth. Twenty-four young white male rats were given a basal ration containing potato starch, casein, margarine, and a standard salt mixture in ad libitum amounts. The rats were divided into four groups, the first of which consumed buttermilk during the entire experiment as a supplement to the basal ration. Another group consumed fresh milk during the entire experiment which lasted ten weeks. The other two groups were given either buttermilk or milk during the first seven weeks and changed to milk or buttermilk during the last three weeks.

Growth was measured as well as food consumption. Growth of all groups appeared to be approximately the same up to the seven weeks' period, and even at the end of ten weeks differences were hardly significant. The two groups consuming milk during the entire experiment or milk in the last three weeks grew a little less well than those consuming buttermilk either during the entire experiment or in the last three weeks. In addition, the buttermilk consumption in the last three or four weeks was somewhat better than the milk consumption. The authors concluded that there was no significant difference between the two kinds of milk, that is fresh or fermented, so far as growth was concerned.

Seborrhea developed in all four groups of animals, beginning about three weeks after the beginning of the experiment, first on the tails and later on the faces of the animals. The authors believed that there was a deficiency in all diets of some dermatitis-preventing factor. No further study of this possible missing factor was made.

In a second study, this time in man, P. Roine and R. Porvari (*Suomen Kemistilehti* **21,** *(B) 43 (1948)*) studied in two groups of experiments the absorption of nitrogen and carotene and of calcium and phosphorus. Milk, either skim or fermented skim was supplied in 1 liter quantities daily in addition to a basal diet containing bread, butter, sugar, marmalade, potato starch, and ascorbic acid.

Two subjects were chosen for study of nitrogen retention and carotene absorption. With regard to the latter, the variations were so great in the carotene content of the feces between the 2 individuals that no conclusions could be drawn. With respect to absorption of nitrogen, that is the percentage in food not appearing in the feces, there was a slightly greater absorption during the period when fermented milk was given than that with fresh milk. The differences were so slight that even though the authors believed that fermented milk improved absorption of nitrogen slightly, it is difficult to attach much significance to the figures.

In the second experiment, 3 individuals were studied, the basal diet being much the same as before except that flour was not consumed in the form of bread. This was done in order to maintain a high phytic acid content in the diet. There were no significant differences in the absorption of calcium and phosphorus between the two periods of skim fresh milk and skim fermented milk consumption. The amount of lactic acid obtained in the fermented milk apparently did not improve the absorption of calcium and phosphorus.

Food Rationing in Finland, 1941 to 1946

Great difficulties are usually met in determining the actual food consumption of a group of people. The inadequacy of the twenty-four hour food intake has been stressed (*Nutrition Reviews* **7**, *242* (*1949*)), especially when food is insufficient. Such sources of food as unrationed materials and those obtained from the black market can rarely be estimated with accuracy. Likewise, determination of the food intake of a population from the ration is open to inaccuracy and difficulty in interpretation. Nevertheless, some useful, interesting information may often be obtained by studying figures obtained in any of these ways.

In Finland, during the years 1941 to 1946, most foods were rationed; in fact potatoes formed the only unrationed food which was available in considerable quantities. P. Roine (*Maataloustieteellinen Aikakauskirja* **20**, *8* (*1948*)), using standard food tables has calculated the composition of the daily rations of food, in terms of calories, protein, fat, minerals, and the common vitamins. Calculations were made for the normal consumer, for children under 1 year of age, children from 1 to 3, 4 to 8, and 9 to 16 years of age, and for the light worker, heavy worker, and very heavy worker. These data were obtained for 1941, again for 1943, and 1945. In addition to the rations actually issued, a quantity of potatoes was added to each ration category, which brought the caloric value approximately to the presumed requirement. In some instances the quantity of potatoes was very large. For example, it was estimated that a very heavy worker consumed 2416 calories daily on the ration; to bring this up to 3800 or 3900, 1500 g. of potatoes were necessary daily.

The food situation appeared to be worse in 1942 and again in 1945, that is, during those years there was thought to be a distinct deficiency, especially in calories, but also other nutrients. The quantities of some of the vitamins appeared to be below the optimum level. Here, however, comparisons were made with the recommended daily allowances of the National Research Council. Such a comparison is hardly fair since these allowances are not minimum requirements. However, the actual ration provided for the people of Finland during these years was probably below minimum requirements for a few of the nutrients. Nevertheless, the availability of foodstuffs not rationed, or those obtained from the black market undoubtedly contributed to the food intake above that issued as the ration. Objective and far more accurate methods must be planned for the future to determine the adequacy of any ration for a given population or group of people.

Letter to the Editor

Dear Sir:

In the review, "Comparative Activity of Carotenoids in Promoting Growth and Vitamin A Storage in Rats," (*Nutrition Reviews* **7**, *180* (*1949*)) you refer to the work of R. M. Johnson and C. A. Baumann, in which they reported that lutein depressed the storage of vitamin A when it was fed with beta-carotene.

I would like to point out to your reviewer a paper published at an earlier date by A. R. Kemmerer, G. S. Fraps, and J. DeMottier (*Arch. Biochem.* **12**, *135* (*1947*)). Our findings were similar to those of Johnson and Baumann. We reported that xanthophylls and also chlorophylls reduced the utilization of carotene for liver storage of vitamin A about 20 per cent. We also pointed out that xanthophylls may in part account for the low utilization of carotene in many vegetables.

A. R. KEMMERER
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Recent Nutrition Book

America's Health—A Report to the Nation. By the National Health Assembly. Harper & Brothers, New York City. 1949. Pp. 388. Price: $4.50.

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